AN MtDNA PERSPECTIVE ON EVOLUTION OF *GILA* (CYPRINIDAE) IN THE COLORADO RIVER BASIN

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Forgotten?

G. cypha

G. robusta

G. elegans

Gila cypha – Life History

- Overall, mostly enigmatic
- Lack of an historic baseline
- Hampered by mainstem/LCR continuum
- Little known about interrelationships among populations
- Deficit will continue to hamper attempts at conserving, recovering and managing the species

Objectives

- Employ mitochondrial DNA to infer genetic interrelationships within and among populations and species
- If possible, identify genetically distinct units so as to assist with developing a management strategy
  - Identify HBC, RTC, BTC
  - Determine levels of variation within each
  - Are there population-specific markers?

Why Mitochondrial DNA?

- Inherited maternally with no recombination
- Evolves 5—10x faster than nuclear genes
- Fewer individuals needed to encapsulate its variance within populations
- Some regions conserved, others quite variable
- Phylogenetic analysis possible

Molecular Genetic Methods

Tissue collection

DNA-Extraction

DNA Amplification (PCR)

Electrophoresis

Modern Technology

- ✓Fluorescent based
- ✓Automated
- ✓Sensitive
- ✓Electronic output

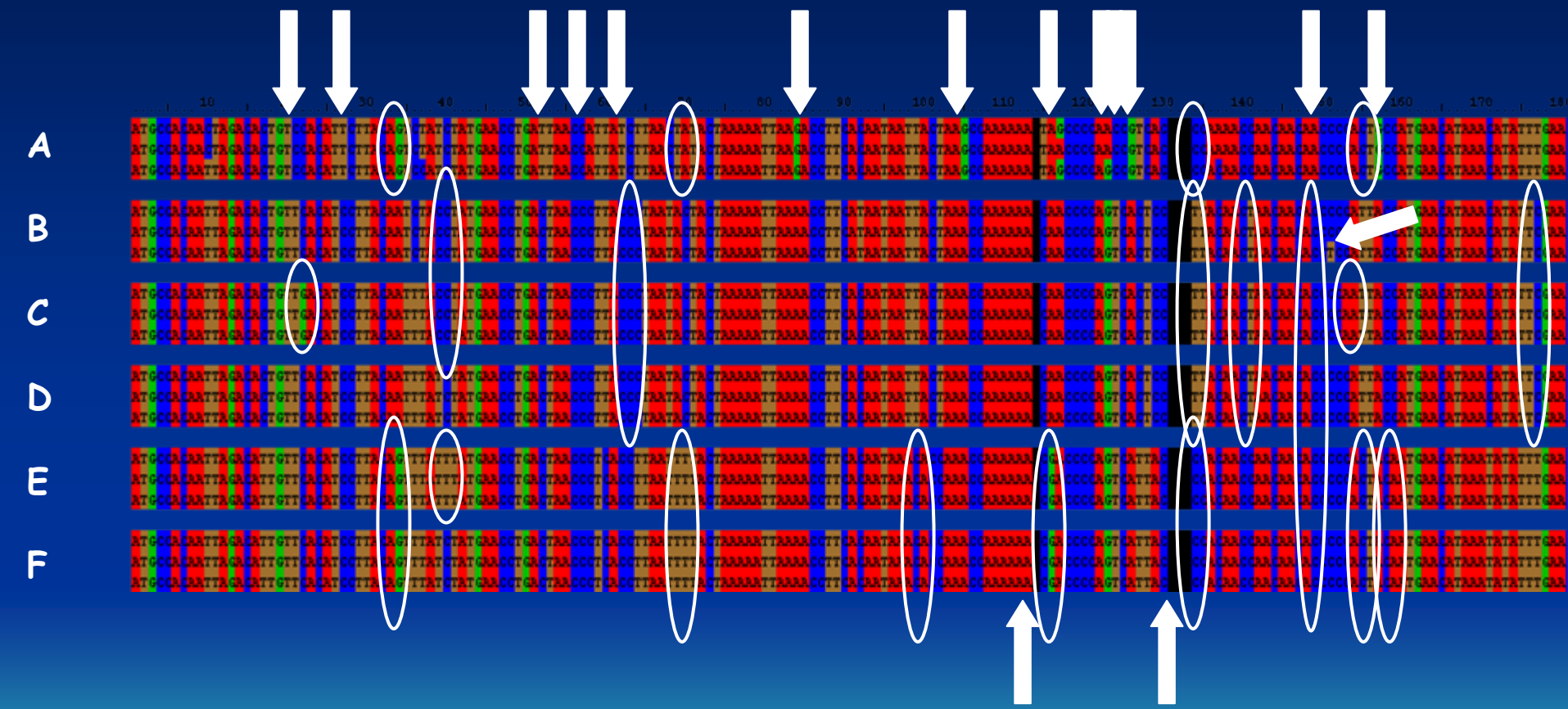
Automated Sequencer

DNA Sequence Data

Each base labeled with different fluorescent dye

A
C
T
G

Elements of Sequence Divergence



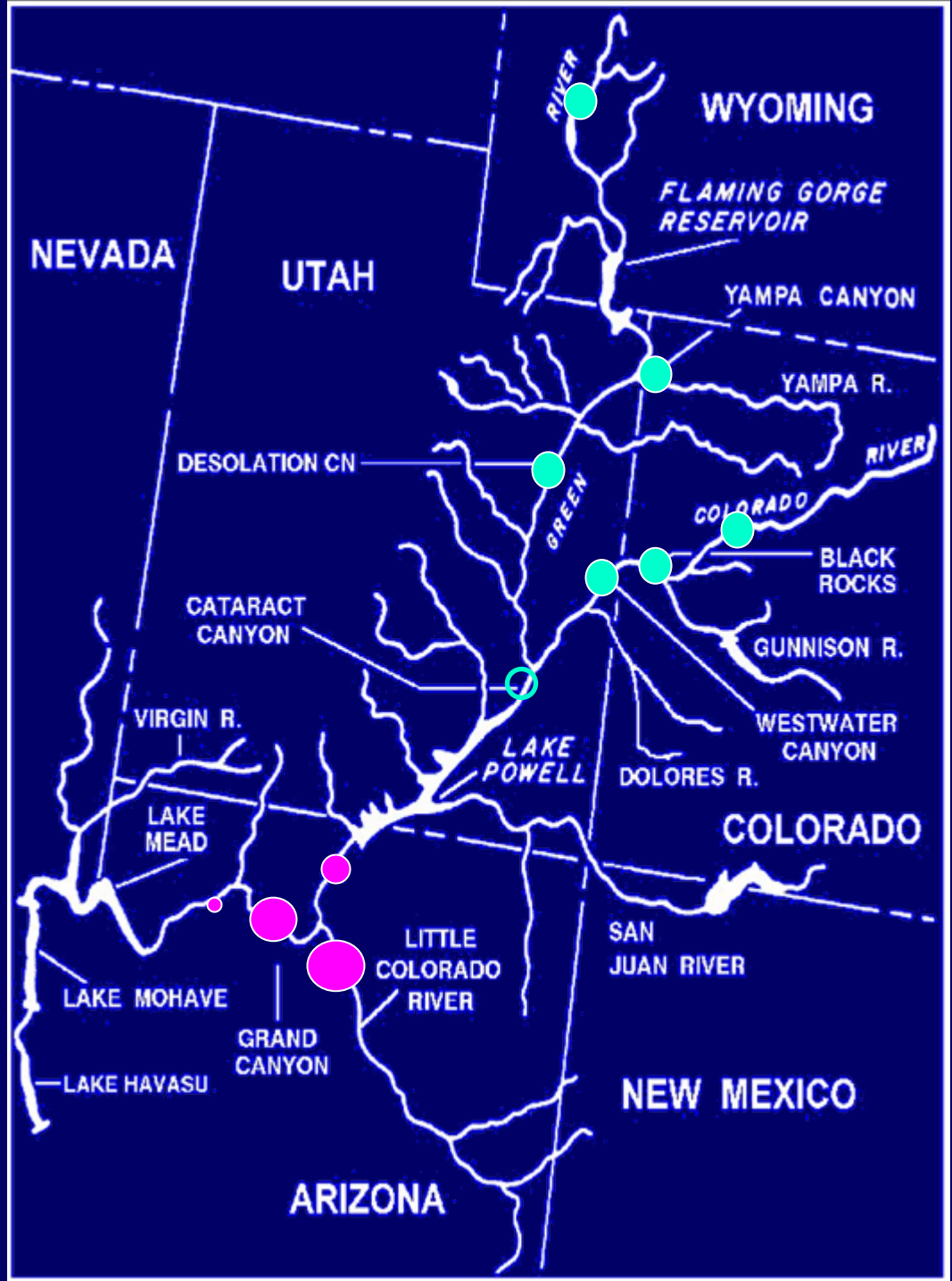
Molecular Markers

- ATP-8 (169 bp)
- ATP-6 (473 bp)
- ND2 (589 bp)
- D-loop (616 bp)

- TOTAL = 1,847 bp
- 12% of mtDNA genome

Upper Basin Sampling

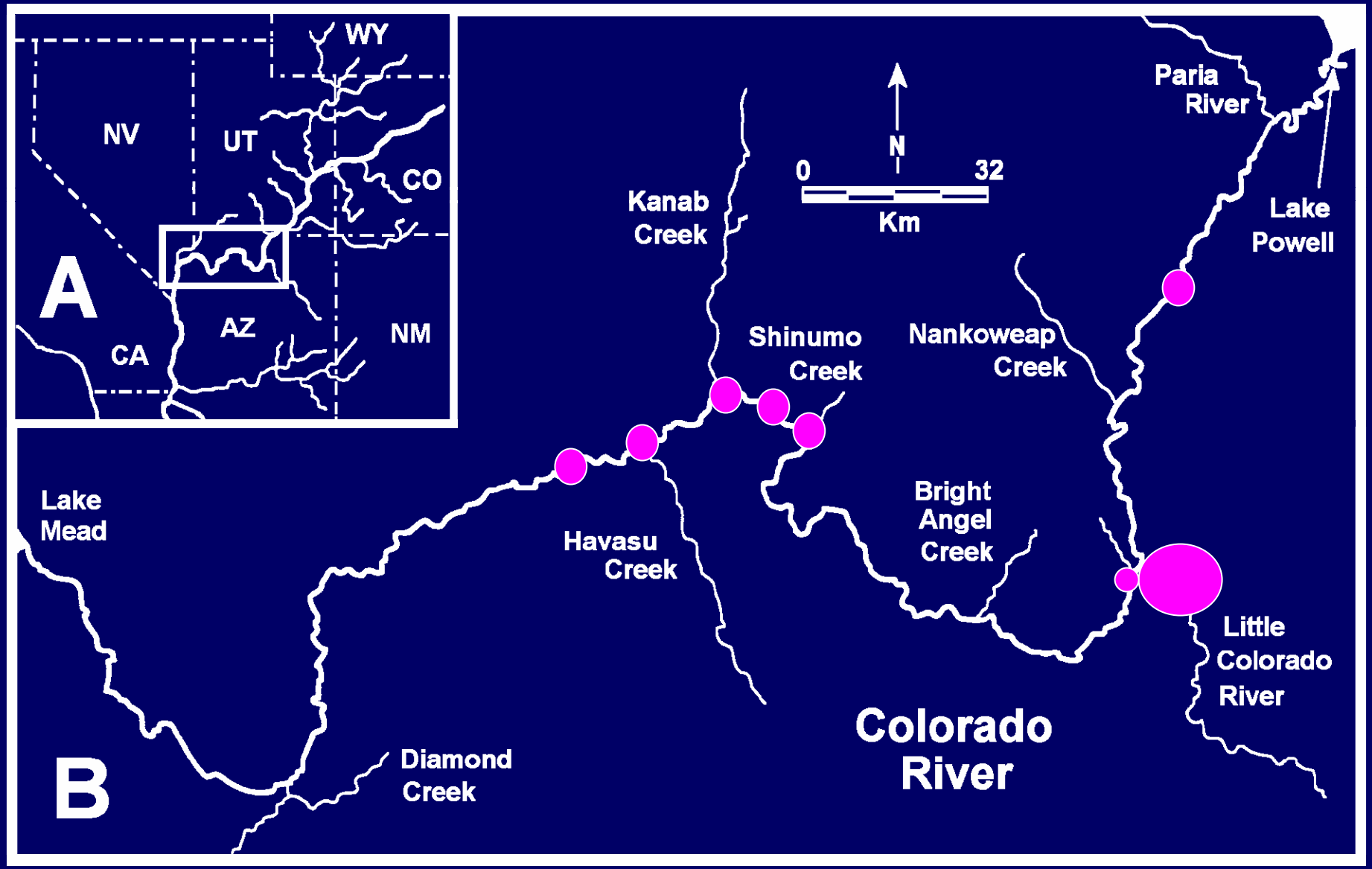
	HBC	RTC
Yampa R	5	30
Desolation Cn	22	22
Black Rocks Cn	16	19
WestWater Cn	20	21
CO River (15 mi.)	--	20
Green R (above FGD)	--	5
TOTAL	63	117


WYOMING
NEVADA
UTAH
COLORADO
NEW MEXICO
ARIZONA
FLAMING GORGE RESERVOIR
YAMPA CANYON
YAMPA R.
DESOLATION CN
GREEN RIVER
COLORADO RIVER
BLACK ROCKS
GUNNISON R.
WESTWATER CANYON
DOLORES R.
CATARACT CANYON
VIRGIN R.
LAKE POWELL
LAKE MEAD
LAKE MOHAVE
LAKE HAVASU
GRAND CANYON
LITTLE COLORADO RIVER
SAN JUAN RIVER

Lower Basin Sampling (HBC)

- 9 locations / 156 HBC
  - 30/40-Mi. Springs 11
  - Little Colorado River 56
  - Shinumo Ck 24
  - Middle Granite Gorge 50
  - Kanab Ck 3
  - Havasu Ck 9
  - Western Grand Cn 3

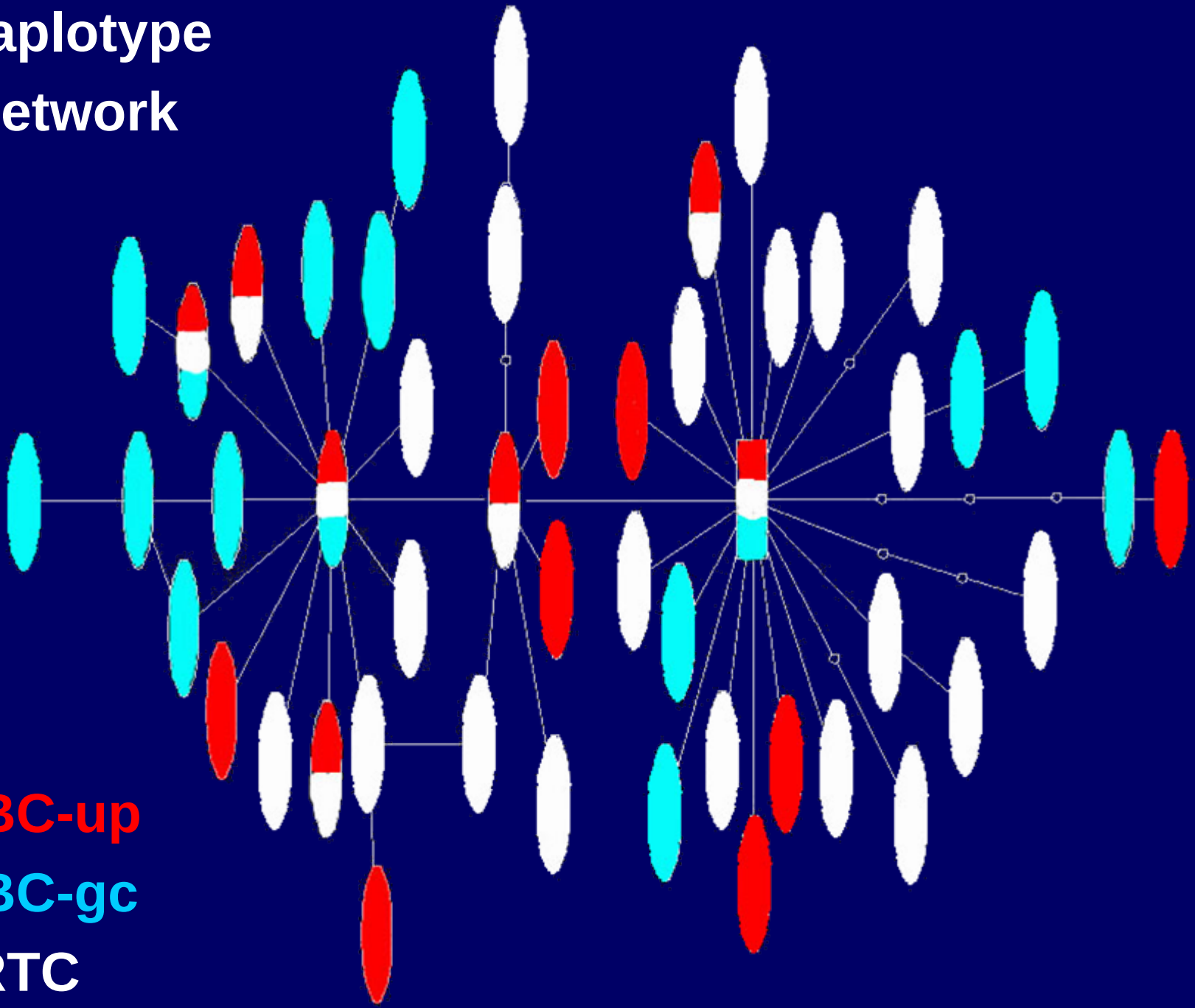
Populations - Grand Canyon



An Echo of *Gila elegans*

- mtDNA imperfect for discerning hybrids
- Overall, 2.5% of individuals (8/331) exhibited BTC haplotypes
  - -- Desolation CN – 9% (2/22)<1 RTC>
  - -- 30/40-mile Sp – 18% (2/11)
  - -- LCR – 3.6% (2/56)
  - -- Randy's Rock (2) – 4% (2/50)
- Of the 3 *Gila* in the Colorado, BTC is ancestral

Haplotype Network



HBC-up

HBC-gc

RTC

Haplotype Distribution

location	#hap	% Total	#ind	% Total	% Group
HBC (lower)	13	27%	128	39%	82%
HBC (upper)	8	16%	10	3%	16%
RTC (upper)	21	43%	35	11%	30%
RTC/HBC	4	8%	17	5%	42/54
3-Shared	3	6%	141	43%	16/67/63

G. Robusta vs G. cypha

- In Upper Basin, ~2/3 of HBC and RTC share haplotypes
- However, RTC found only in UB
- In Grand Canyon, 16% of individuals share haplotypes with RTC and upper basin HBC
- Unique haplotypes at all locations in each basin are few in number and primarily restricted to singletons

% Sequence Divergence

- Negligible sequence divergence between HBC and RTC (circa 0.09%)
- No differences between upper/lower basin HBC
- BTC differs from other *Gila* at 4.9% (± 0.5)
- *Ptychocheilus lucius* differs from *Gila* at 5.6% (± 0.6)

Co-Mingled Haplotypes

- Caused by 'Ancestral Polymorphism'
  - originally present in ancestor population
  - retained in current populations
  - not enough time has elapsed for them to sort out
- AP often links populations even if they are geographically isolated
- 'Lineage sorting' changes frequency of AP
  - unique mutations occur within isolated populations and move to fixation

Preliminary Conclusions (1)

- Ancestral polymorphism links populations within and across basins
- Upper basin
  - more unique haplotypes, but
  - higher frequency of shared haplotypes
- Lower basin
  - fewer unique haplotypes, but
  - basin-specific haplotypes more frequent

Preliminary Conclusions (2)

- Haplotype divergence small
  - mtDNA cannot ascertain relationships among populations
- Apparent regional fixation of haplotypes should be interpreted cautiously as it is sample-size dependent
- Limitations are imposed by evolutionary rate(s) of molecular markers employed

Havasu Creek at confluence of Colorado River in Grand Cn

Msat Distribution -- CRE